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ABSTRACT

Process for the Determination of In Vitro Amplified Nucleic Acids

A process for the qualitative and quantitative determination of at least one in vitro amplified nucleic acid in a sealed reaction chamber,

- wherein during or subsequent to the amplification of the nucleic acid at least one substance (probe) is present which interacts with the nucleic acid to be detected;
- wherein spectroscopically measurable parameters of said substance (probe) are subject to variation, creating a measurable signal;
- wherein the sample to be measured is exposed to the action of a gradient capable of at least partially denaturing nucleic acids.
- with detection of the measurable parameter undergoing variation through the action of he gradient; and
- the entire amplification reaction, including qualitative and quantitative determination, may be carried out in a sealed reaction chamber (measuring compartment) without intermittent opening,

permitting to automatically operate the diagnostic method of DNA and RNA amplification in quantitative and quantitative fashion on large series of samples. The method is based on a comparison of copy number and sequence of DNA or RNA to be analyzed and an internal standard, using a time/temperature gradient. Detection is conducted using optical processes in homogenous or heterogenous phase and does

not require any separation techniques. The amplified reaction batch may remain sealed even during and subsequent to analysis. Thus, for example, disposal is possible, completely avoiding the risk of contamination by amplified reaction batches. The analyzed samples may be archived for prolonged periods of time and, if necessary, analyzed repeatedly. By subsequent separation using temperature gel electrophoresis, optional variants, e.g., for the purpose of sequencing, may be produced preparatively.